International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Antihypertensive Potency of Goat Milk Yoghurt Supplemented by Probiotic and Roselle Extract

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Abstract

Goat milk has several advantages such as milk whiter color, the size of the fat globules are smaller so it is more easily absorbed by the body and can be consumed by people with lactose intolerance, but the weakness is goaty odor. Processing technology can decrease the goaty odor such as fermentation. Probiotic bacteria also can improve the functional properties of fermented goat milk because of proteolytic activities, such as antihypertensive function. Roselle flower has antioxidant capacity that can improve quality of yoghurt. The aim of the research was to evaluate antihypertensive potency that showed by angiotensin I-converting enzyme (ACE) inhibitory activities from goat milk yoghurt supplemented by probiotic and roselle extract. Treatments were (1) goat milk yoghurt, (2) goat milk yoghurt + probiotic *L. acidophillus* IIA-2B4, (3) goat milk yoghurt + Roselle extract, and (4) goat milk yoghurt + probiotic *L. acidophillus* IIA-2B4 + extract of Roselle. The result showed that probiotic *L. acidophilus* IIA-2B4 contributed in peptide fragments separation of yoghurt. Antihypertensive activities demonstrated that goat milk yoghurt was able to inhibit the angiotensin converting enzyme (ACE) activity an enzyme that plays a role in the renin angiotensin aldosterone system as a function of blood pressure regulation in human body. The ACE inhibitor activity of yoghurt probiotic roselle was higher than the yoghurt probiotic. The goat milk yoghurt supplemented by probiotic and roselle extract was met the Indonesian national standard of yoghurt and safe to be consumed.

Keywords: Antihypertensive potency; goat milk yoghurt; probiotic.

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1. Introduction

Hypertension, defined as the systolic blood pressure above 140 mmHg and/or diastolic pressure above 90 mmHg, is one of the major chronic diseases affecting 30% of the adult population in the world. Angiotensin I-converting enzyme (ACE) is an enzyme that increase blood pressure then causing hypertension. ACE is a Dipeptidyl carboxypeptidase that converts the inactive decapeptide angiotensin I into a potent and active vasoconstrictor, by deactivating bradykinin as a vasodilating agent [1]. Inhibition of ACE gives an anti-hypertensive effect by reducing blood pressure. Antihypertensive activity is able to inhibit the angiotensin converting enzyme (ACE) activity an enzyme that plays a role in the renin angiotensin aldosterone system as a function of blood pressure regulation in human body. Some sources of protein-containing foods such as fish, gelatin, soy and milk proteins are reported to contain active peptides which can serve as an ACE inhibitor. Casocinin and lactocin in an ACE inhibitor result of enzymatic hydrolysis during fermentation of casein [2].

Goat milk has several advantages over cow milk. Despite its advantages, goat milk has limitations in term of short shelf life and goaty odor. To overcome these limitations, in this research, the goat milk was processed into yoghurt by fermentation process. Many dairy starter cultures are highly proteolytic. Formation of bioactive peptides can, thus, be expected during the manufacture of fermented dairy products. In fact, the release of different bioactive peptides from milk proteins through microbial proteolysis. Yoghurt contains animal protein but it also contains bioactive peptides. Bioactive peptides have an important role in the physiological functions and regulatory functions in the body [3]. One function of these peptides is as inhibitors of the enzyme activity of ACE (Angiotensin Converting Enzyme) which is responsible for the occurrence of hypertension. The aim of the research was to evaluate Angiotensin-i converting enzyme (ACE) inhibitory activities that contribute to antihypertensive potency from goat milk yoghurt supplemented by probiotic and roselle extract.

2. Materials and Methods

2.1. Extraction of roselle (Hibiscus sabdariffa L)

Dried rosella flowers finely milled flour, sieved using a sieve size of 60 mesh. Roselle flour dissolved in water with a ratio of 20 g: 100 mL which is then pasteurized at a temperature of 63-65 ° C for 30 minutes [3].

2.2. Goat milk yoghurt production

Goat milk was heated at a temperature of 75-80 ° C for 30 minutes, then let cool until the temperature reaches 37 ° C. Yogurt cultures (*Streptococcus thermophillus* RRAM-01 and *Lactobacillus bulgaricus* RRAM-01) and *Lactobacillus achidophillus* IIA-2B4 was added as much as 3% of the volume of goat milk, incubated at 37 ° C for 18 hours until a coagulation (plain yoghurt). Followed by the addition of roselle extract as much as 1% of the volume of goat milk [4].

2.3. Analysis of Peptide Fragments

The content of peptide fragments were determined by the method based on the Lowry [5]. 0.05 mL of yoghurt has been separated below 10 kDa and was added with 0.095 mL distilled water. The samples were diluted with

the reagent Lowry. 0.5 mL folin of was added and allowed to stand for 30 minutes, then analyzed by spectrophotometer with a wavelength of 750 nm.

2.4. Antihypertensive analysis

Antihypertensive analysis was done by using ACE inhibitor based on Hayes and his colleagues [6]. The enzyme substrate used was hippuryl-histidyl-L-leucine (HHL). Samples tested were taken as 6 mL added to 50 mL HHL with 7.6 mM and then incubated at 37 °C for 5 minutes using a water bath. Then added 20 mL of ACE and incubated for 5 minutes at a temperature of 30 °C.

The reaction was stopped by addition of 250 mL of 0.5 M HCl, and 1.5 mL ethyl acetate. Sample solution was then centrifuged at 2500 rpm for 15 minutes, 1 mL of the supernatant was separated. Drying using a block heater to a temperature of 100 °C for 10 minutes. The sample was cooled at room temperature. Furthermore, the absorbance reading using a spectrophotometer with wavelength of 228 nm. The activities of inhibition was calculated by formulae:

Inhibitor activity (%) = 100 – [100 x ((C – D)/(A- B))]

Notes :

A: Absorbance ACE without the addition of the sample

B: Absorbance sample

C: Absorbance of the sample was added ACE

D: Absorbance without the addition of ACE and samples

2.5. Quality analysis of yoghurt

The quality analysis of yoghurt was done at PT Saraswanti Indo Genetech, the Indonesian molecular biotechnology company, an accredited laboratory for quality control analysis. The methods used by company were national and international procedure standard.

2.6. Statistical data analysis

The experimental design used in this study was completely randomized design (CRD) factorial design [7]. Treatments were (1) goat milk yoghurt, (2) goat milk yoghurt + probiotic *L. acidophillus* IIA-2B4, (3) goat milk yoghurt + Roselle extract, and (4) goat milk yoghurt + probiotic *L. acidophillus* IIA-2B4 + extract of Roselle. Each treatment was performed three repetitions.

3. Result and Discussion

To improve its quality, a probiotic bacterium isolated from Indonesian cattle, *Lactobacillus acidophillus* IIA-2B4 [8], and roselle extract were added to the yoghurt. The high population of LAB in yoghurt with addition of *L. acidophilus* IIA-2B4 with or without roselle extract (range of population was 9 log CFU / mL) were proportional to the acidity of the product that promotes higher viscosity compared to the control. Proximate analysis revealed that additions of *L. acidophilus* IIA-2B4 and/or roselle extract significantly reduced fat content, while ash content was significantly increased by the treatments (data not shown). The results of the analysis of peptide fragments shows that the addition of *L. acidophillus* IIA-2B4 and roselle extract in yoghurt could increase the activity of proteolysis in yoghurt. Therefore, testing the content of peptides in yoghurt which had undergone separation below 10 kDa by using the method of Lowry. The results obtained are presented in Table 1.

Table 1: Peptide content of goat milk yoghurt

Yoghurt type	Peptide content (µg/mL)
Yoghurt	11.46 ± 0.01^{c}
Yoghurt Roselle	11.41 ± 0.01^{c}
Yoghurt Probiotic	12.10 ± 0.04^{a}
Yoghurt Probiotic Roselle	12.80 ± 0.02^{b}

Data represents means ± standard error from 3 samples

Values in the same column followed by a different superscript alphabet shows significance differences ($P<0.05$).

The test results showed that the yoghurt is added *L. acidophilus* IIA-2B4 contains peptides were high compared with yoghurt (control) and with the addition of rosella flower extract (Table 1). This is due to the *probiotic L. acidophillus* IIA-2B4 can increase the activity of protein proteolysis [9]. In addition the test results showed that the extract of Roselle contribute in the process of proteolysis when combined with the use of probiotics. Fermented milk peptides have dual activity. The peptides may function as an antimicrobial and inhibitor of angiotensin-converting enzyme (ACE) that can be used for prevention and treatment of hypertension [10]. Therefore, analysis of antihypertensives in yoghurt with addition of *L. acidophillus* IIA-2B4 or without extract Roselle to confirm this.

Angiotensin Converting Enzyme (ACE) is an enzyme that plays a role in regulating blood pressure within the Renin-angiotensin system (RAS) [11]. ACE catalyzes the conversion of decapeptide angiotensin I to angiotensin II octapeptide. ACE works by releasing dipeptide on the terminal C of angiotensin I to produce angiotensin II and hippuric acid [12]. Angiotensin II stimulates the synthesis and secretion of aldosterone which increases blood pressure by increasing sodium retention in the distal tubule [13]. Working ACE can have an impact in the high blood pressure. ACE inhibition is one way to reduce the risk of high blood pressure.

The process of fermentation of milk can result in the ability to inhibit the enzyme ACE [11]. It is caused by the

bacterial culture fermentation process break down proteins into peptides having ACE inhibiting properties. Peptides in milk have the ability to inhibit the ACE by 52% with IC_{50} value of 12:41 mg/mL [4]. The content of peptides in yoghurt probiotic and yoghurt probiotic roselle bigger than the yoghurt and yoghurt roselle (Table 1). Therefore, the analysis of antihypertensive only done on yoghurt probiotic and yoghurt probiotic roselle. The concentration of peptide needed to inhibit 50% of the work ACE called IC_{50}. Yoghurt ability in inhibiting ACE enzyme and IC_{50} can be seen in Table 2.

Table 2: Activities inhibits the enzyme ACE on probiotic yoghurt and probiotic yoghurt roselle

Goat milk yoghurt	ACE inhibition (%)	IC_{50} (µg/mL)
Yoghurt probiotic	20.40 ± 1.68a	21.48 ± 0.01c
Yoghurt probiotic roselle	37.76 ± 1.43b	13.87 ± 0.01d

IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the ACE activity

Data represents means ± standard error from 3 samples

Values in the same column followed by a different superscript alphabet shows significance differences ($P<0.05$)

The peptide produced in the fermentation process plays a role in ACE inhibitory activity. The results show that ability in inhibiting ACE of Yoghurt Probiotic Roselle is greater than the Yoghurt Probiotic. Differences in the ability to inhibit the ACE is one of them caused by the content of peptide Yoghurt Probiotic Roselle higher than the Yoghurt Probiotic (Table 2). This is consistent with Donkor and his colleagues [4] which states that the peptides are produced from the fermentation process plays a role in inhibition of ACE activity in the yogurt due to the combination of *L. bulgaricus* and *S. thermophillus,* inhibition of ACE in yoghurt increased by the addition of probiotics [4]. Probiotic plays a role in this research was *L. acidophilus* IIA-2B4.

Table 2 shows that the addition of roselle flower extract on probiotic yoghurt affect ($P <0.05$) the ability to inhibit ACE and the addition of roselle flower extract is used significantly ($P <0.05$) IC_{50} values. This caused roselle extract cumulatively contribute in inhibiting ACE. Antihypertensive activity in roselle extract due to the flavonoid phytochemical components. The mechanism of flavonoids in inhibiting ACE is to inhibit the formation of angiotensin II [14]. The content of flavonoids in roselle extract the greatest role in the inhibition of ACE is anthocyanin [15].

As a functional food, beside should contain beneficial function for human health, the food also should met quality and food safety standard. Because of that, the research continued by analysis of quality characteristics of goat yoghurt probiotic roselle that has highest antiphypertensive activity. Table 3 shows the quality of goat yoghurt probiotic roselle. The heavy metals such as lead (Pb), cadmium (Cd), Tin (Sn), mercury (Hg)and arsenic (As) were not found in goat yoghurt probiotic roselle. Toxin such as aflatoxin M1 also was not detected in yoghurt. Microbiological analysis showed that goat yoghurt probiotic roselle was not contaminated by

pathogenic bacteria such as *Salmonell*a sp and *Listeria monocytogenes*. Energy content of yoghurt support as 65.71 kkal / 100 g. All quality characteristics of goat yoghurt probiotic roselle was accordance with SNI (Indonesia National Standard) of yoghurt and safe to be consumed.

Table 3: Quality characteristics of Goat Yoghurt Probiotic Roselle

Parameter	**Unit**	**Result**	**Limit of detection**	**Method**
Total energy	kkal/ 100 g	65.71	-	Calculation
Pb	ppm	Not detected	0.00900	18-8-31/MU/SMM-SIG.
Cd	ppm	Not detected	0.00011	18-8-31/MU/SMM-SIG.
Sn	ppm	Not detected	3.01000	18-8-31/MU/SMM-SIG.
Hg	ppm	Not detected	0.00400	18-8-31/MU/SMM-SIG.
As	ppm	Not detected	0.00800	18-8-31/MU/SMM-SIG.
Coliform	APM/mL	< 3	-	SNI 2981-2009
Salmonella sp	/ 25 mL	negative	-	SNI 2981-2009
Listeria monocytogenes	/ 25 mL	negative	-	
Sodium	mg / 100 mL	47.57	-	18-8-31/MU/SMM-SIG
Kalium	mg / 100 mL	146.08	-	18-8-31/MU/SMM-SIG
Total solid without fat	%	8.03		SN 01-3713-1995
Lactic acid	ppm	14223.22	-	18-5-32/MU/SMM-SIG, HPLC
Total sugar	%	1.32	-	SNI 01-2892-1992
Aflatoxin M1	ppb	Not detected	0.01000	18-5-58/MU/SMM-SIG, HPLC

Notes : SNI = National Indonesian Standard

4. Conclusion

Goat milk yoghurt supplemented by probiotic *L. acidophilus* IIA-2B4 with or without roselle extract in goat milk yoghurt have antihypertensive potency as functional food. Peptide fragments increased and contributed to the antihypertensive properties. The ACE inhibitor activity or antihypertensive potency of yoghurt probiotic roselle was greater than the yoghurt probiotic. In addition, the quality of goat milk yoghurt supplemented by probiotic and roselle extract was in accordance with Indonesian national standard of yoghurt.

Acknowledgements

The authors would like to thank to Ministry of Research, Technology and Higher Education–Republic of Indonesia which has provided the research fund through the Research Grant 2014 and continued by IBIKK Grant 2016 No 079/SP2H/PPM/DRPM/II/2016 date 17 February 2016.

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